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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Application of: | Karatzas *et al.* | Confirmation No.: | 1912 |
| Serial No.: | 09/040,518 | Art Unit: | 1632 |
| Filed: | March 17, 1998 | Examiner: | FALK, ANNE MARIE |
| For: | PRODUCTION OF BIOFILAMENTS IN TRANSGENIC ANIMALS | Attorney Docket No.: | 9529-009-999 (06632/011001) |

**SECOND DECLARATION OF DR. COSTAS N. KARATZAS UNDER 37 C.F.R. § 1.132**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, COSTAS N. KARATZAS, declare that:

1. I am an inventor, along with Jeffrey D. Turner and Anthoula Lazaris Karatzas, of the subject matter claimed in U.S. Application No. 09/040,518 (the "'518 application") filed March 17, 1998.

2. I am presently employed by Nexia Biotechnologies, Inc. ("Nexia"), 1000 St. Charles Avenue, Block B, Vaudreuil-Dorion, QC, J7V 8P5 Canada, where I hold the position of Senior Vice-President, Research & Development. Nexia is the assignee of the '518 application.

3. I understand that '518 application currently claims nucleic acid molecules comprising a nucleotide sequence encoding a polypeptide and a regulatory sequence that directs expression of a polypeptide in milk-producing cells of a ruminant. The regulatory sequence is operably linked to the nucleotide sequence and the polypeptide comprises a

plurality of repeat motifs as present in dragline silk produced by *Nephila clavipes* or *Araneus diadematus* and a leader sequence that enables secretion of the biofilament polypeptide into milk of a ruminant when the nucleic acid is expressed in milk-producing cells of the ruminant. I understand that other currently pending claims are directed towards a transgenic female ruminant comprising germline and somatic cells that comprise the nucleic acid molecule as described above, wherein the ruminant secretes a biofilament polypeptide into milk. Moreover, I understand that additional pending claims are directed towards methods of producing a biofilament polypeptide by isolating the biofilament polypeptide from the milk of a ruminant expressing the polypeptide, or by isolating the biofilament polypeptide from the medium of a cell culture that expresses the polypeptide.

4. To date, I or other researchers working under my supervision have generated more then 100 transgenic female ruminants using nucleic acid molecules comprising the whey acidic protein (WAP) promoter or β-casein promoter and biofilament polypeptide-encoding sequences as described in the '518 application.

5. I believe, and those of skill in the art are likely to believe, that the concentrations of spider silk biofilament polypeptides expressed in the milk of transgenic ruminants that are described in the following paragraphs are more than sufficient to permit the isolation of the biofilament polypeptides from the milk produced by the transgenic female ruminants.

6. For example, using the methods described in the specification coupled with the knowledge available to those of skill in art at the time the '518 application was filed, I or researchers working under my supervision generated founder goats by pronuclear microinjection that were transgenic for the spider silk biofilament polypeptide ADF-3 gene under control of the WAP promoter and leader sequences as described in the '518 application. Female transgenic offspring of a founder goat were produced and hormonally induced into lactation. Twenty liters of milk taken from nine goats, measured to contain approximately one gram of ADF-3 biofilament polypeptide, was used to successfully isolate ADF-3 from the milk in quantity and purity suitable for use in, for example, spinning fibers.

7. As another example, seven female transgenic progeny of goats generated by pronuclear microinjection were obtained that expressed approximately 20-400 milligrams of MaSpI per liter of milk. The expression of the spider silk biofilament polypeptide MaSpI was under control of the WAP promoter. The concentration of MaSpI secreted into the milk is more than sufficient to permit its isolation.

8. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Costas N. Karatzas

November 23d, 2004

Date